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Research Article

Genetic diversity of grapevine (*Vitis vinifera* L.) as revealed by ISSR markers

Rezq Basheer-Salimia · Arwa Mujahed

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Abstract The main goal of this study was to determine the genetic diversity among 36 grape cultivars grown in Palestine by using ISSR-polymerase chain reaction (PCR) fingerprints. Among the tested primers, 17 produced reasonable amplification products with high intensity and pattern stability.

A total of 57 DNA fragments (loci) separated by electrophoresis on agarose gels were detected and they ranged in size, from 150 to 900 bp. Out of these fragments, 55 (88%) were polymorphic and 2 (3.5%) monomorphic. Our results also revealed an average of 3.1 loci per primer. A minimum of 1 and maximum of 10 DNA fragments were obtained (S-17, #820 and #841) and (S-31) primers, respectively. Therefore, the later primer (S-31) is considered to be the most powerful primer among the tested ones.

The genetic distance matrix showed an average distance range of between 0.05 and 0.76. The maximum genetic distance value of 0.76 (24% similarity) was exhibited between the (Shami and Marawi.Hamadani.Adi) as well as (Bairuti and Marawi.Hamadani.Adi) genotypes. On the other hand, the lowest genetic distance of 0.05 (95% similarity) was exhibited between (Jandali.Tawel.Mofarad and Jandali. Kurawi.Mlzlz) along with (Shami.Aswad and Shami.mtartash. mlwn) genotypes. Furthermore, the UPGMA dendrogram generally clusters the grape cultivars into eight major clusters in addition to an isolated genotype. Based on these figures, the cultivars tested in this study could be characterized by large divergence at the DNA level. This is taking the assumption that our region has a very rich and varied clonal grape genetic structure.

R. Basheer-Salimia (✉)
Department of Plant Production and Protection, College of Agriculture, Hebron University
e-mail: rezqbasheer@hebron.edu

A. Mujahed
Department of Applied Biology, College of Applied Science, Palestine Polytechnic University

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Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest known fruit trees cultivated in Palestine mainly for wine, table fruit, juice, and raisins. Since 14 centuries (establishment of Islamic civilization), its plantation dedicated only on table grape seeded-cultivars and it was growing mainly at the southern part of West-Bank especially Hebron and Bethlehem areas (Sultan 2005), in which the geographical and environmental conditions are suitable for growing high-quality fruits. Currently, it is considered as the second important fruit crop after olive in terms of areas covered, economic returns, and social values (Basheer-Salimia 2015a; MOA 2017). Lately, its plantation starts to expand into the northern regions focusing mainly on seedless grape.

The long domestication history with numerous cultivars and further introduction of new cultivars especially from the surrounding countries has resulted in ambiguity in the description and nomenclature of grape cultivars (Basheer-Salimia 2015b). Furthermore, many cultivars were also subjected either to genetic deterioration and/or to disappearance leading thereby to lose a great pool of grape genetic materials. Indeed, such problems might relate to different biotic and abiotic stresses, especially the climate change which characterizes the region lately where temperature and drought is dramatically increased (Basheer-Salimia and Ward 2014). Therefore, determination of genetic variability and proper cultivar identification in grapevine would be of major importance for any future improvement programs and germplasm preservation as well.

In general, morphological (based mainly on traditional field evaluation) and/or DNA molecular markers are commonly used for detection and analyzing of genetic variation

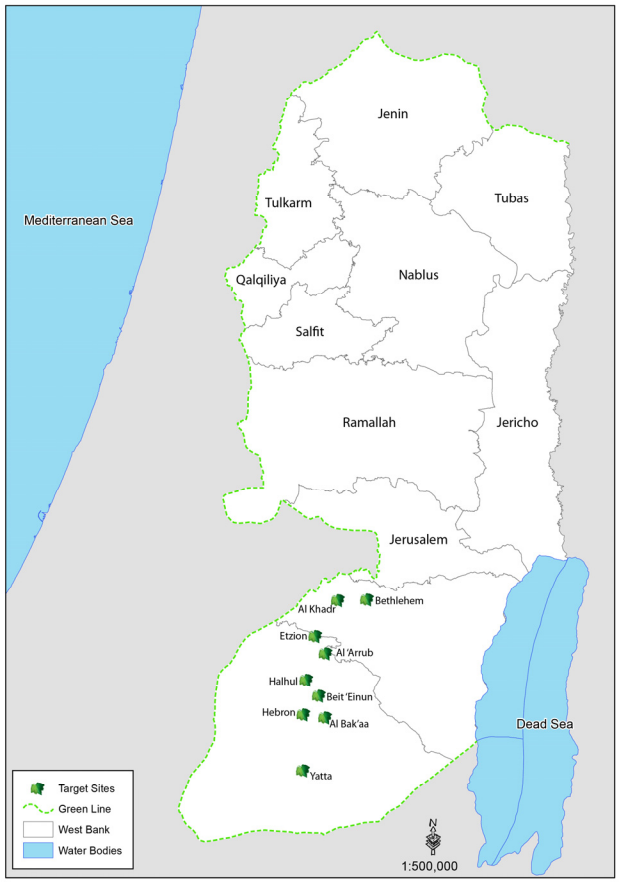

Fig. 1 Map showing the grapevine collection sites

among fruit tree species (Basheer-Salimia et al. 2012. In fact, characterizations based on morphological criteria's are considered as time-consuming, laborious and error-prone due to environmental factors, which poses great challenges (Sripholtaen et al. 2016), in addition to its high variability across years and locations (Lima et al. 2002; Achtak et al. 2009). Alternatively, DNA-based markers proved to be powerful tools to estimate genetic diversity as well as genotype identity of many horticultural species including grape (Herrera et al. 2002; Bahurupe et al. 2013). In fact, molecular markers offer numerous advantages over conventional morphological based approach, since they proved to be: (1) allow direct comparison of different genetic material independent of environmental influences (Weising et al. 1995); (2) stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell (Chittora et al. 2015); (3) accurate and rapid genotype identification especially in vegetatively propagated plants species (Depnath 2009), such as grape; and (4) not confounded by the environment, pleiotropic, and epistatic effects (Agarwal et al. 2008).

Comparing with the other common DNA markers; Inter Simple Sequence Repeat markers (ISSRs) have been used successfully in many fruit tree species because it's simple, fast, high stability, no prior requirement of sequence information, cost effectiveness and versatility of markers (Qian et al. 2001; Reddy et al. 2002; Choudhary et al., 2014). In grapes, numerous DNA-based markers have been conducted toward characterization of grape species in the Mediterranean region (Riaz et al. 2018); however rare studies have been found in the literature on Palestinian grapes. In the present work, we have employed ISSRs technique to determine the number of genetically different grapevine cultivars that were actually exists in Palestine; to infer possible cases of synonymy and homonymy; and to evaluate the genetic relationships of the characterized cultivars.

Materials and Methods

Plant materials

Healthy grape leaves were collected from the middle-region of the newly growing shoots from 36 assumed cultivars (>50 years) throughout Hebron and Bethlehem districts (Fig. 1).

DNA extraction and purification

Two medium - healthy, young leaves of each assumed cultivar were ground with pestle and mortar using liquid nitrogen to fine powder. A weight of 100 mg of leaf powder of each cultivar was transferred into a 1.5 mL eppendrof tube and mixed with, 400 µL of buffer AP1 and 4 µL of RNase A stock solution (100 mg/mL) were added to the tube, and the mixture was vigorously vortexed, incubated at 65°C, and mixed 2-3 times during incubation by inverting. A quantity of 130 µL of buffer AP2 was added to the lysate, which was mixed, incubated on ice for 5 minutes, and then centrifuged at 20,000 x g. The supernatant was applied to the QIA-shredder Mini Spin Column and it was carefully centrifuged at 20,000 x g for 2 minutes so as not to disturb the pellet. The flow-through fraction (liquid) was transferred to a new 2 mL eppendrof tube without disturbing the cell-debris pellet and a quantity of 1.5 volumes of buffer AP3/E was added to the cleared lysate and mixed by pipetting. A quantity of 650 µL of the mixture was applied to the DNeasy Mini Spin Column placed in a 2 mL collection tube which was centrifuged at 6,000 x g while its flow-through was discarded. The rest of the mixture was applied as aforementioned. Subsequently, the DNeasy Mini Spin Column was placed in a new 2 mL collection tube, and 500 µL of buffer AW was added to it. The tube was centrifuged at 6,000 x g for 1 min, and the flow-through was discarded, reusing the collection tube in the next step. A total volume of 500 µL of the same buffer was used once more with centrifuging at 20,000 x g for 2 min. The DNeasy Mini Spin Column was transferred to a 1.5 mL eppendrof tube and a quantity of 30 µL of buffer AE was added twice with a separation time of at least 5 min between them. Before storing at 20°C, the tube was centrifuged at full speed for 1 min.

Estimation of DNA quantification

DNA quality and quantity was tested on 0.8% agarose gel electrophoresis using Lambda DNA as a standard. Other measurements also done for DNA concentration and purity using spectrophotometer. Final concentration of DNA was adjusted to 50 ng/µl.

Inter Simple Sequence Repeats (ISSR) / PCR reaction mixture and program

Twenty ISSR primers (Table 1) were used for the amplification of random ISSR banding patterns according to Sabir et al. (2009). PCR reactions were carried out in a 25 µl volume mixture containing: 5 µl of a total DNA (50 ng), 4 µl primer (5 µM), 2 µl dNTPs (200 mM) (Fermentas), 2.5 µl Taq buffer (10X), 2 µl magnesium chloride (25 mM) and 1.5 U of Taq DNA polymerase (Hy Labs). Consequently, DNA was amplified by PCR on a Peltier Thermal Cycler-200 (MJ Research. Inc, Watertown, MA) and the PCR program was: 1 cycle, 94°C (3 min); 40 cycles, 94°C (1 min), 54°C (1 min), 72 (2 min) 1 cycle, 72°C (7 min), and then cooling down to 4°C.

Table 1 List of the used ISSR primers

No.	Primer name	Primer sequence (5`→3`)
1	#4	5`GAG AGA GAG AGA GAG AYG3`
2	#811	GAG AGA GAG AGA GAG AC
3	S-17	GAG AGA GAG AGA GAG AT
4	S-19	GAG AGA GAG AGA GAG AA
5	#9	ACA CAC ACA CAC ACA CYG
6	S-13	ACA CAC ACA CAC ACA CYC
7	S-14	AGA GAG AGA GAG AGA GT
8	S-16	AGA GAG AGA GAG AGA GG
9	S-27	BDB CAC ACA CAC ACA CA
10	S-31	AGA GAG AGA GAG AGA GVC
11	UBC-855	ACA CAC ACA CAC ACA CYA
12	#890	VHV GTG TGT GTG TGT GT
13	#841	GAG AGA GAG AGA GAG AYC
14	S-30	HVH TGT GTG TGT GTG TG
15	#840	GAG AGA GAG AGA GAG AYT
16	#836	AGA GAG AGA GAG AGA GYA
17	#826	ACA CAC ACA CAC ACA CC
18.	#825	ACA CAC ACA CAC ACA CT
19	#820	GTG TGT GTG TGT GTG TC
20	#818	CAC ACA CAC ACA CAC AG

Where Y: (C,T); B: (C,G,T); D: (A,G,T); H: (A,T,C) V: (A,C,G); and R: (A,G).

ISSR-Gel processing

Amplified products (25 µl) were mixed with 5 µl of orange gel loading buffer and analyzed by electrophoresis in 3% agarose gels (Hy Labs) in 1X TAE buffer at 4 volt/cm for 4h as well as detected by staining with ethidium bromide (Sigma). A 100 bp DNA ladder was used as standard marker (Fermentas). Consequently, amplicons were visualized and photographed black and white on Polaroid type film with UV trans-illuminator (ImageMaster®VDS). DNA bands were scored (1) for presence and (0) for absence for each primer-

genotype combination. Only reliable and clear bands were scored for the estimation of genetic similarity.

ISSR Data analysis

Data matrix was utilized to generate genetic similarity data among genotypes using. Jacquard's similarity coefficient formula as the following:

$$S_{ij,Jaccard} = \frac{n_{11}}{n_{11} + n_{01} + n_{10}}$$

Where *nxy* is the number of characters that have state *x* in individual *i* and state *y* in individual *j*. Un-weighted pair group method using arithmetic averages (UPGMA) (Schluter and Harris 2006) phenogram was then calculated from the Jaccard's similarity using fingerprint analysis with missing data (FAMD) software version 1.108 beta. Tree view software (Win32) version 1.6.6 was used to visualize the resulted trees. All trees presented in this study were mid-pointed.

Results

Genetic variations and relatedness among grapevine accessions based on ISSR

Among the 20 tested primers used to investigate the pattern of genetic variation between 36 accessions of grape grown at the southern region of West-Bank, Palestine; 17 primers produced reasonable amplification products with high intensity and pattern stability (Table 2), whereas, only 2 primers (S-27 and #841) exhibited ambiguous, light, and non-clear complex amplification products and primer #840 produce no amplification, and therefore were excluded from our analysis.

A total of 57 DNA fragments (loci) separated by electrophoresis on agarose gels, were detected (Table 2), ranging in size from 150 to 900 bp (Fig. 2). Of these fragments, 55 (88%) were polymerphic and 2 (3.5%) were monomorphic. Our results also revealed an average of 3.1 loci per primer (Table 2). A minimum of 1 and a maximum of 10 DNA fragments were obtained using (S-17, #820 and #841) and (S-31) primers, respectively (Table 2). There-

Table 2 Analysis of the polymorphism obtained with ISSR markers

		Approximate band size (bp).					
primers name	Total No. ISSR bands	Min.	Max.	Mono-morphic band	Poly-morphic bands	Poly-morphic (%)	Primer case
# 9	6	200	800	0	6	100%	Included
S-13	2	300	400	0	2	100%	Included
# 4	5	150	400	0	5	100%	Included
S-17	1	400	400	0	1	100%	Included
S-19	3	250	600	0	3	100%	Included
S-14	3	250	500	0	3	100%	Included
S-16	5	150	400	0	5	100%	Included
S-27	4	250	450	4	0	0%	Excluded
S-31	10	180	800	2	8	80%	Included
# 818	5	250	700	0	5	100%	Included
# 820	1	300	300	0	1	100%	Included
# 825	2	300	400	0	2	100%	Included
# 826	2	400	450	0	2	100%	Included
#836	2	600	700	0	2	100%	Included
S-30	2	300	400	0	2	100%	Included
#841	1	350	350	1	0	0%	Excluded
#890	2	300	400	0	2	100%	Included
UBC-855	1	300	300	0	1	100%	Included
# 811	5	280	900	0	5	100%	Included
Total	62			7	55	88%	

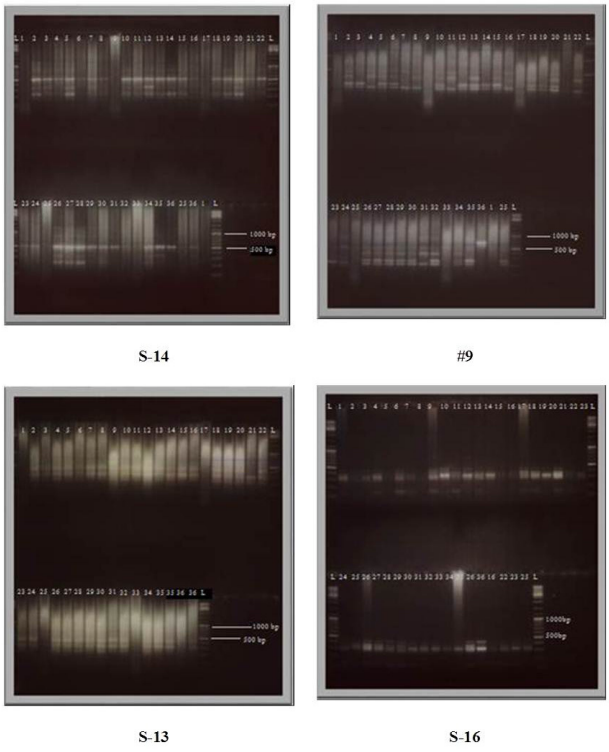

Fig. 2 Examples of ISSR banding patterns generated in Palestinian Grapevine cultivars using S-14, #9, S-13, and S-16 primers (100 bp ladder)

fore, the later primer (S-31) is considered as the most powerful primer among the tested once (Fig. 2). The maximum percentage of polymorphic markers was 100% with 17 primers (Table 2), however, the minimum percentage was 80% with only one primer (S-31).

Genetic distances

The data matrix size analyzed included 2232 entries, 1288 of which were for present loci (1) and 944 for absent loci (0). Accordingly, the Jaccard coefficient was calculated and presented in table 7. The genetic distance matrix showed an average distance range from 0.05 to 0.76 with a mean of 0.405. The maximum genetic distance value of 0.76 (24% similarity) was exhibited between (Shami and Marawi. Hamadani.Adi) as well as (Bairuti and Marawi.Hamadani.Adi) genotypes, whereas the lowest genetic distance of 0.05 (95% similarity) was exhibited between (Jandali.Tawel.Mofarad and Jandali.Kurawi.Mlzlz) along with (Shami.Aswad and Shami.mtartash.mlwn) genotypes.

UPGMA analysis

UPGMA dendrogram clustered the grape genotypes into eight major clusters in addition to an isolated genotype

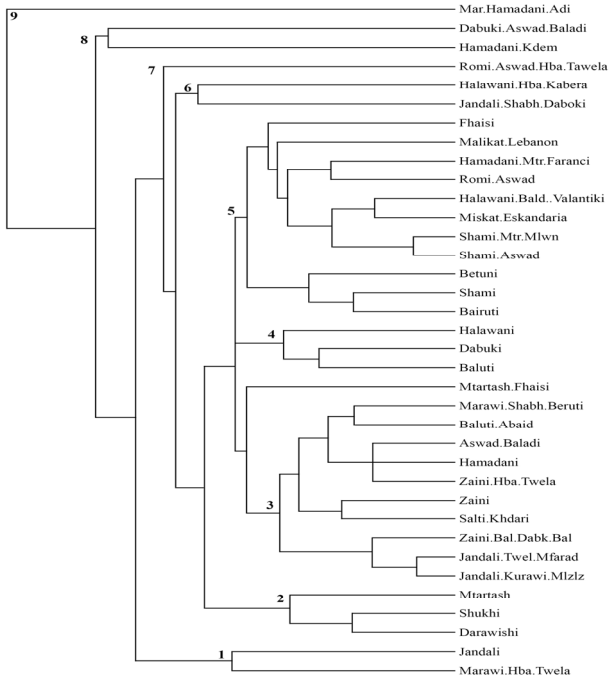

Fig. 3 Dendrogram of 36 local Palestinian Grapes constructed by UPGMA based on ISSR banding patterns

Marawi.Hamadani.Adi as a distinctive one (Fig. 3). Cluster "1", consists of only two genotypes: Marawi.Habe.Tawela and Jandali. Cluster "2" contains Darawishi and Shukhi genotypes which both are related to Mtartash. Cluster "3" is further divided into two highly related small groups (Jandali.Kurawi.Mlzlz, Jandali.Tawel.Mafrod, and Zaini.Baladi. Dabuki.Baladi) and (Sulti.Khdari, Zaini, Zaini.Habe.Tawela, Hamadani, Aswad.Baladi, Baluti.Abiad and Marawi.Shabh. Bairuti), in which all also are connected into Mtartash. Fhaisi cultivar. Cluster "4" composed of (Baluti and Dabuki) related to Halawani.

Cluster "5" is sub-divided into small groups including ("Bairuti and Shami" related to Betuni), (highly related Shami.Aswad and Shami.Mtartash.Mlwn), (Miskat.El. Eskandaria and Halawani.Baladi.Valantiki), (Romi.Aswad and Hamadani.Ma´tar.Faranci), and another two related individual genotypes Malikt.Libnan and Fhaisi.

Cluster "6" consists of Jandali.Shabh.Dabuki and Halawani. Habe.Kabera. Cluster “7” composed of only one genotype Romi.Aswad.Habe.Tawela. Finally, cluster "8" consists of Hamadani.Kadem and Dabuki.Aswad.Baladi.

Discussion

Grape (*Vitis vinifera* L.) is known for its wide morphological and genetic diversity and the existence of large

Table 3 Jaccard's distance index generated for the 36 local Palestinian grapevines' ISSR data

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1.Mar.Hamadani.Adi	0.00																																			
2.Shami.Mtr.Mlwn	0.67	0.00																																		
3.Halawani.Bald.Valntiki	0.64	0.16	0.00																																	
4.Hamadani.Matr.Faranc	0.58	0.20	0.21	0.00																																
5.Shami.Aswad	0.68	0.05	0.16	0.15	0.00																															
6.Romi.Aswad	0.62	0.30	0.31	0.17	0.30	0.00																														
7.Shukhi	0.68	0.27	0.24	0.23	0.28	0.21	0.00																													
8.Malikt.Libnan	0.73	0.20	0.26	0.24	0.21	0.26	0.20	0.00																												
9.Jandali	0.51	0.49	0.43	0.32	0.46	0.38	0.44	0.54	0.00																											
10.Miskat.Eskandaria	0.62	0.20	0.11	0.15	0.16	0.26	0.20	0.29	0.38	0.00																										
11.Mtartash.Fhaisi	0.64	0.32	0.33	0.24	0.29	0.37	0.35	0.29	0.49	0.29	0.00																									
12.Halawani	0.64	0.28	0.29	0.32	0.29	0.37	0.27	0.33	0.49	0.24	0.28	0.00																								
13.Zaini	0.65	0.37	0.38	0.26	0.34	0.31	0.33	0.34	0.45	0.30	0.22	0.30	0.00																							
14.Betuni	0.68	0.31	0.33	0.27	0.28	0.33	0.30	0.28	0.48	0.28	0.31	0.23	0.18	0.00																						
15.Shami	0.76	0.29	0.34	0.33	0.26	0.38	0.35	0.21	0.59	0.33	0.33	0.33	0.23	0.16	0.00																					
16.Bairuti	0.76	0.33	0.38	0.40	0.33	0.41	0.32	0.21	0.59	0.37	0.33	0.36	0.31	0.24	0.14	0.00																				
17.Dabuki	0.59	0.33	0.30	0.24	0.29	0.34	0.32	0.37	0.42	0.21	0.29	0.20	0.23	0.24	0.33	0.40	0.00																			
18.Baluti	0.60	0.36	0.33	0.32	0.33	0.33	0.39	0.40	0.32	0.28	0.43	0.28	0.30	0.23	0.36	0.40	0.19	0.00																		
19.Salti.Khdari	0.63	0.29	0.26	0.21	0.26	0.27	0.28	0.33	0.41	0.17	0.29	0.25	0.16	0.24	0.26	0.37	0.17	0.24	0.00																	
20.Fhaisi	0.64	0.33	0.22	0.25	0.30	0.27	0.24	0.26	0.39	0.21	0.29	0.29	0.31	0.29	0.30	0.30	0.30	0.29	0.26	0.00																
21.Hamadani	0.63	0.36	0.30	0.21	0.33	0.27	0.24	0.26	0.41	0.26	0.25	0.33	0.20	0.28	0.26	0.30	0.26	0.33	0.14	0.22	0.00															
22.Darawishi	0.71	0.38	0.36	0.31	0.39	0.29	0.14	0.28	0.48	0.32	0.38	0.35	0.29	0.30	0.32	0.28	0.36	0.42	0.32	0.29	0.20	0.00														
23.Mtartash	0.65	0.47	0.42	0.33	0.45	0.31	0.25	0.38	0.43	0.38	0.40	0.44	0.35	0.36	0.38	0.34	0.41	0.41	0.34	0.31	0.23	0.21	0.00													
24.Marawi.Haba.Twela	0.53	0.47	0.37	0.31	0.44	0.33	0.34	0.40	0.31	0.32	0.43	0.43	0.40	0.42	0.50	0.50	0.36	0.35	0.32	0.37	0.28	0.38	0.37	0.00												
25.Zaini.Bal.Dabk.Bal	0.63	0.30	0.31	0.22	0.27	0.24	0.26	0.30	0.42	0.23	0.26	0.30	0.24	0.26	0.31	0.34	0.27	0.30	0.20	0.31	0.16	0.33	0.31	0.33	0.00											
26.Jandali.Twel.Mafrod	0.65	0.38	0.40	0.31	0.36	0.33	0.30	0.39	0.36	0.32	0.35	0.35	0.33	0.34	0.39	0.39	0.36	0.31	0.28	0.36	0.24	0.38	0.33	0.38	0.09	0.00										
27.Jandali.Kurawi.Mlzlz	0.65	0.38	0.40	0.31	0.36	0.33	0.34	0.43	0.36	0.32	0.35	0.31	0.29	0.30	0.35	0.39	0.36	0.31	0.24	0.33	0.24	0.38	0.33	0.42	0.14	0.05	0.00									
28.Aswad.Baladi	0.63	0.40	0.38	0.25	0.37	0.30	0.35	0.37	0.41	0.33	0.33	0.33	0.20	0.28	0.30	0.37	0.30	0.33	0.22	0.30	0.14	0.28	0.34	0.36	0.20	0.24	0.20	0.00								
29.Zaini.Haba.Twela	0.63	0.40	0.38	0.25	0.37	0.30	0.32	0.33	0.38	0.33	0.29	0.33	0.23	0.32	0.33	0.37	0.30	0.33	0.22	0.30	0.10	0.28	0.30	0.32	0.16	0.16	0.16	0.10	0.00							
30.Marawi.Shabh.Beruti	0.65	0.33	0.35	0.22	0.34	0.24	0.26	0.27	0.45	0.30	0.33	0.33	0.21	0.26	0.23	0.27	0.30	0.37	0.20	0.27	0.11	0.18	0.24	0.36	0.24	0.33	0.29	0.16	0.20	0.00						
31.Baluti.Abiad	0.67	0.27	0.32	0.23	0.27	0.24	0.33	0.27	0.47	0.35	0.34	0.34	0.29	0.30	0.28	0.31	0.31	0.34	0.24	0.32	0.20	0.30	0.32	0.41	0.25	0.33	0.30	0.20	0.20	0.13	0.00					
32.Dabuki.aswad.baladi	0.66	0.61	0.53	0.54	0.59	0.51	0.56	0.59	0.50	0.51	0.67	0.58	0.57	0.53	0.60	0.64	0.51	0.42	0.50	0.53	0.50	0.56	0.59	0.37	0.53	0.56	0.56	0.50	0.50	0.53	0.55	0.00				
33.Halawani.H.Kabera	0.71	0.36	0.34	0.36	0.33	0.38	0.39	0.44	0.46	0.33	0.40	0.40	0.41	0.39	0.44	0.40	0.41	0.37	0.37	0.38	0.40	0.43	0.38	0.48	0.27	0.28	0.28	0.40	0.40	0.41	0.31	0.59	0.00			
34.Jandali.Shabh.Dabuki	0.70	0.42	0.37	0.39	0.40	0.40	0.38	0.40	0.45	0.36	0.42	0.42	0.40	0.41	0.36	0.32	0.40	0.39	0.36	0.28	0.32	0.38	0.40	0.43	0.36	0.34	0.30	0.32	0.32	0.36	0.33	0.58	0.36	0.00		
35.Hamadani.Kadem	0.67	0.59	0.50	0.43	0.56	0.53	0.50	0.60	0.47	0.44	0.59	0.62	0.55	0.60	0.64	0.67	0.53	0.61	0.48	0.54	0.48	0.50	0.53	0.48	0.55	0.57	0.57	0.51	0.51	0.51	0.56	0.48	0.56	0.59	0.00	
36.Romi.Aswad.H.Twela	0.67	0.51	0.46	0.37	0.49	0.42	0.43	0.52	0.47	0.41	0.48	0.44	0.41	0.47	0.51	0.54	0.41	0.45	0.37	0.43	0.33	0.36	0.42	0.36	0.38	0.40	0.36	0.25	0.29	0.38	0.35	0.48	0.38	0.40	0.45	0.00

number of cultivars (Santos et al. 2013). Therefore, determination of genetic variability and proper cultivar identification would be of major importance for any future breeding programs and germplasm collection (Jing et al. 2013). In the present study, the level of polymorphism among 36 grapevine (*Vitis venifera* L.) accessions commonly grown in Palestine were estimated using DNA-based marker technique, in which 20 ISSR primers (dinucleotide repeats, commonly used for grape identification; Wang et al. 1994; Moreno et al. 1998), were used to identify and discriminate the Palestinian grapes. Many authors stated that, few ISSR primers (5 primers) were needed to generate diagnostic and reproducible fingerprint profiles and therefore distinguishing between the varieties (Moreno et al. 1998).

Of these primers screened initially on 36 grapevine cultivars, 18 primers yielded an altered interval of polymorphism from 1 to 8. However, the other two primers (S-27 and #841) were excluded from the analysis since they produced no polymorphism. Reddy et al. (2002) and Herrera et al. (2002) reported that some primers were more efficient in recognizing a complementary site in the plant genome. Comparing with Herrera and others (2002), our results revealed higher polymorphic bands (55 bands) using 18 primers relating to 40 bands using 11 primers (which are parallel to our examined primers). Interestingly, primer #820 that showed only one band in Merlot cultivar cultivated in Chile also revealed the same number of bands (1 band) with our Bairuti genotype so these genotypes maybe related, however, further investigation is needed to support this assumption.

Among the evaluated 20 primers; 6 were GA repeats (the maximum), 5 were AC repeats, 4 were AG repeats, 3 were GT repeats, and 2 were CA repeats. Remarkably, 3 motifs (AG) of nucleotide repeats were realized, with 100% polymorphism (Table 3). Consequently, the primers revealed significant differences in resolving polymorphism, in which the primers containing (GT)n repeats and (CA)n repeats were the most polymorphic. The same is true for the average number of bands per primer.

Thomas and others (1993) found the repeats GA and GT as the most highly represented in the Vitis genome. Further, Dhanorkar and others (2005) recorded the repeats AC as more common among ISSR primers selected by using Indian grapes in a more recent study.

Contradictory to the fact that AT motifs in the plant kingdom have generally been approved as the most plentiful repeat (Casasoli et al. 2001), here, in grapes our results revealed 4 AG motifs among the 20 tested primers which confirm the results of Dhanorkar et al. (2005) who also registered 6 AG motifs in Indian grapes. Consequently, the relative abundance of nucleotide repeats in the grapevine genome indicates differences between different studies conducted on *Vitis* species.

The size of amplified fragments varied from 150 bp to 900 bp. This interval was narrower than the results obtained by Dhanorkar, and others (2005) and Sabir and others (2009), who reported fragments between (300 bp and 1500 bp) and (300 bp and 2500 bp), for different grape cultivars in India and Turkey, respectively. Indeed, the narrower amplified fragments exhibited by our cultivars might related to the smaller and restricted cultivated areas (Hebron and Bethlehem regions, about 2000 Km^2) in which our grapevines are grown comparing with the very large studied areas of India and Turkey.

The percentage of polymorphism revealed in the present analysis is much higher than those reported by Moreno et al. (1998); Dhanorkar et al. (2005); Sabir et al. (2009). This could be attributed to the pre-selection of our primers for their abilities to generate clear and polymorphic-band-patterns and/or the intra-varietal differences as it is presented in our study rather than the inter-varietal differences among the examined cultivars.

The values of the genetic distances ranged from 0.05 for the most closely related cultivars (Jandali.Tawel.Mofarad and Jandali.Kurawi.Mlzlz) and (Shami.Mtaratsh.Mlwn and Shami.Aswad) to 0.76 for the most distantly related cultivars (Marawi.Hamadani.Adi and Shami) and (Marawi.Hamadani. Adi and Bairuti). These results confirm that these cultivars could be the same cultivar but with different names (synonyms). Vignani (1996) set a precedent that closely related individuals could be considered under a common name. UPGMA dendrogram (Fig. 3) clustered the grape genotypes into eight major clusters including 2, 3, 11, 3, 11, 2, 1 and 2 genotypes, respectively. In addition to that, cluster 9 (Marawi.Hamadani.Adi) was isolated as a distinctive genotype. Interestingly, some examined cultivars showed high similarities with each other such as (Jandali.Tawel.mafrod and Jandali.Kurawi.Mlzlz, by 95%; Shami.Aswad and Shami. Mtartash.Mlwn, by also 95%), which might assumed that these cultivars are highly correlated and therefore might be the same cultivars with different names. The most distant cultivars were between (Marawi.Hamadani.Adi and Shami, by 24%) and (Marawi.Hamadini.Adi and Bairuti, by 24%). Therefore, these cultivars could be useful and interesting grape genotypes for any future breeding program in Palestine. In addition, since ISSR represents an efficient tool for estimating the genetic variability and the genetic relationships among our examined grapevine genotypes therefore, ISSR

markers could be a useful technique for grapevine genotype identification's and characterizations.

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